Effects of Carbon Dioxide and Distilled Water on the Carbon Dioxide Output of Frog-skin.

Slifer

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The Effects of Carbon Dioxide and Distilled Water
on the
Carbon Dioxide Output of Frog-skin.

Eleanor H. Slifer

A Thesis submitted to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Science.

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Frog-skin is a relatively simple tissue consisting of a few layers of cells with small glands scattered just below and extending up thru the surface layer. It is easily removed from the animal and, when properly treated, will remain in a living condition for many hours. Such a substance has been extensively used and offers many interesting possibilities for experimentation. At the suggestion of Doctor Bodine a study of the rate at which it produces carbon dioxide was undertaken.

The method used for the determination of carbon dioxide was a modification of that developed by Osterhout and Haas (1) and consisted of the immersion of the skin in a vial containing Ringer's solution colored with phenol red and the comparison of the color changes occurring in this solution with the colors of a series of standards containing known amounts of carbon dioxide.

Five Pyrex vials with ground-glass stoppers and each with a measured capacity very close to 20 c.c. were chosen. These, as well as the rest of the apparatus, were frequently boiled with distilled water in order to remove any traces of acid or alkali which might disturb the results. 5 c.c. of Ringer's solution, colored with phenol red, were placed in each vial. Two, sometimes three, of these were used as receptacles in which to test the carbon dioxide output of the skin. Into the remaining vials carbon dioxide saturated water, in definite quantities, was introduced so as to give a graded series of standards.

The frog were was then pithed. The white skin from the abdomen, throat and ventral surface of the legs was employed because it interfered less than did the pigmented portions in the judgment of any color changes later occurring in the liquid in which it was to be placed. As soon as the skin had been removed it was spread out

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on a clean glass plate, covered with Ringer's solution, and scraped very gently in order to remove any adherent blood vessels or muscle tissue. Then it was cut into two, three or more pieces, according to the requirements of the experiment, and kept until needed in an open beaker containing Ringer's solution. A piece of skin was then removed from the beaker, laid flat on a piece of filter paper and pressed gently with another bit of paper in order that the amount of moisture carried into the vial with the skin be as uniform as possible in each experiment. The skin was then transferred immediately to the vial in which it was to be tested. The time was recorded and the solution carefully observed in order to detect any changes taking place in its color.

At frequent intervals the vial was shaken so that any gases produced might be distributed more rapidly between the liquid and the air above it. This was done quickly and with the top of the vial held by the tips of the fingers so as to prevent, as far as possible, any variations in the temperature which might be caused by the warmth of the hand. All color comparisons were made against a white background and with the light from a north window, the vials being slanted in such a way that slight differences in color could best be detected. When the color of the solution containing the skin accorded exactly with that of the first standard the time was noted. The same thing was done when the other standards were reached. During the work, as special problems presented themselves, minor variations were made in the method and these will be described more fully below.

The experiments extended over February, March, April and May and approximately sixty pieces of skin from twenty-three different frogs were tested. Seven R. catesbiana and one R. palustris were used the remainder being, for the most part, R. pipiens or closely

related species. The temperature during the course of the work varies from 19 to 25 C. but in the case of any particular experiment remained constant. No experiment was allowed to extend over more than six hours and this probably eliminated bacterial growth as a source of error.

The first thing necessary to know was whether or not frog-skin in Ringer's solution does produce an acid. That it does can be clearly and easily demonstrated in the presence of an indicator such as phenol red. The acid formed under these conditions is quite volatile and is readily driven off on heating. For example, if two samples of Ringer's solution, colored WITH PHENOL RED, ONE OF WHICH HAS BEEN USED IN AN EXPERIMENT with frog-skin and become quite yellow, are boiled, each will present, after a short time, exactly the SAME coloration. The acid expelled by heat is carbon dioxide. Were any non-volatile acids given off by the skin the solution would not regain its original color on boiling.

It is reasonable to suppose that a tissue such as frog-skin produces carbon dioxide in amounts directly proportional to its weight. That this is the case was demonstrated repeatedly during these experiments. Two pieces of skin of equal weight and from the same animal, when treated in exactly the same way, give approximately identical results. Fig. I shows, graphically, how closely the rates of different bits of skin from the same frog resemble one another. Pieces of skin from two different frogs, however, do not produce carbon dioxide at exactly similar rates. Individual and probably specific differences, as well as seasonal variations, play a part.

THE A skin when first placed in the vial produced carbon dioxide at a rate so rapid as to be quite striking. This outburst or "gush" was soon followed by a gradual and steady decline which continued as

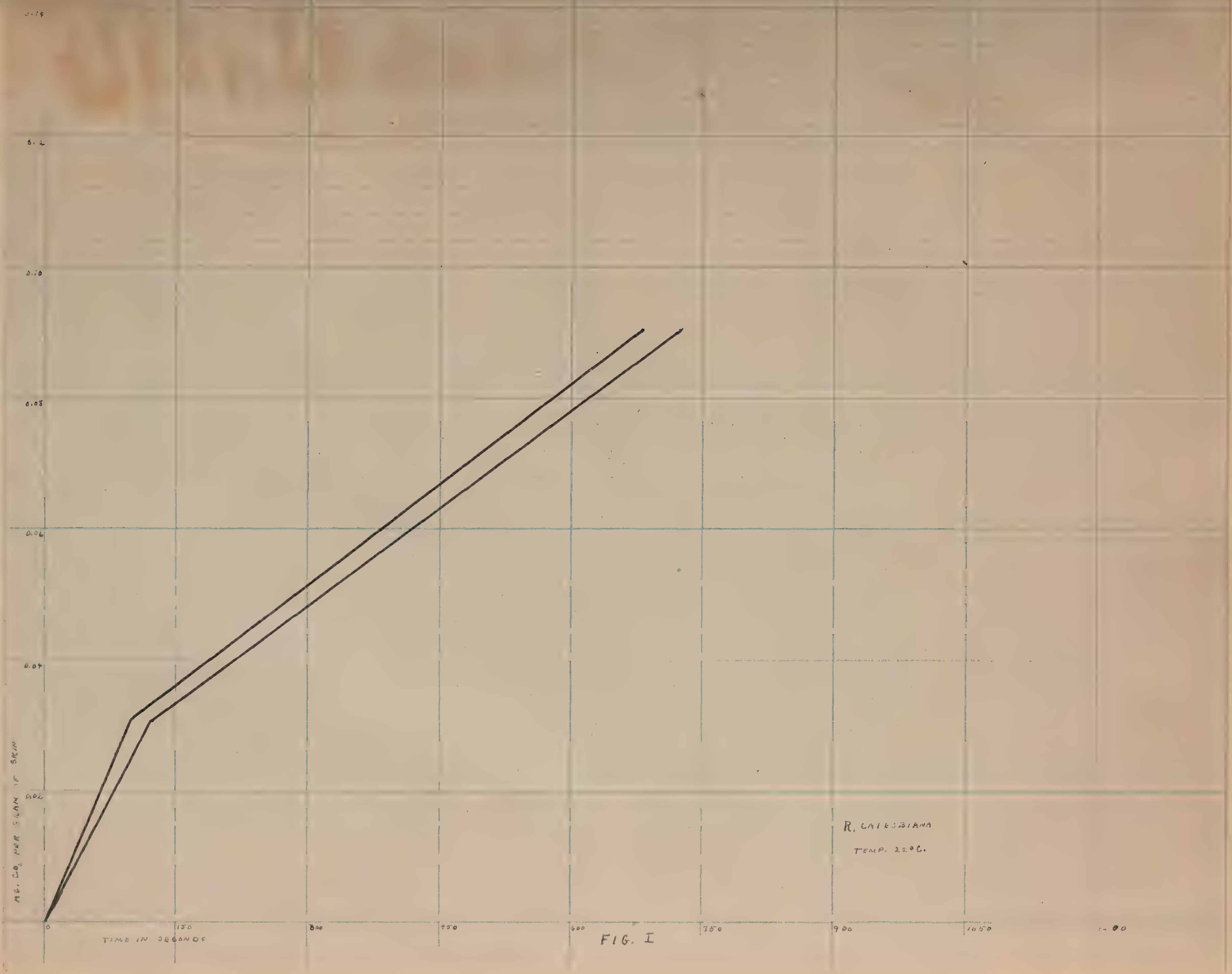
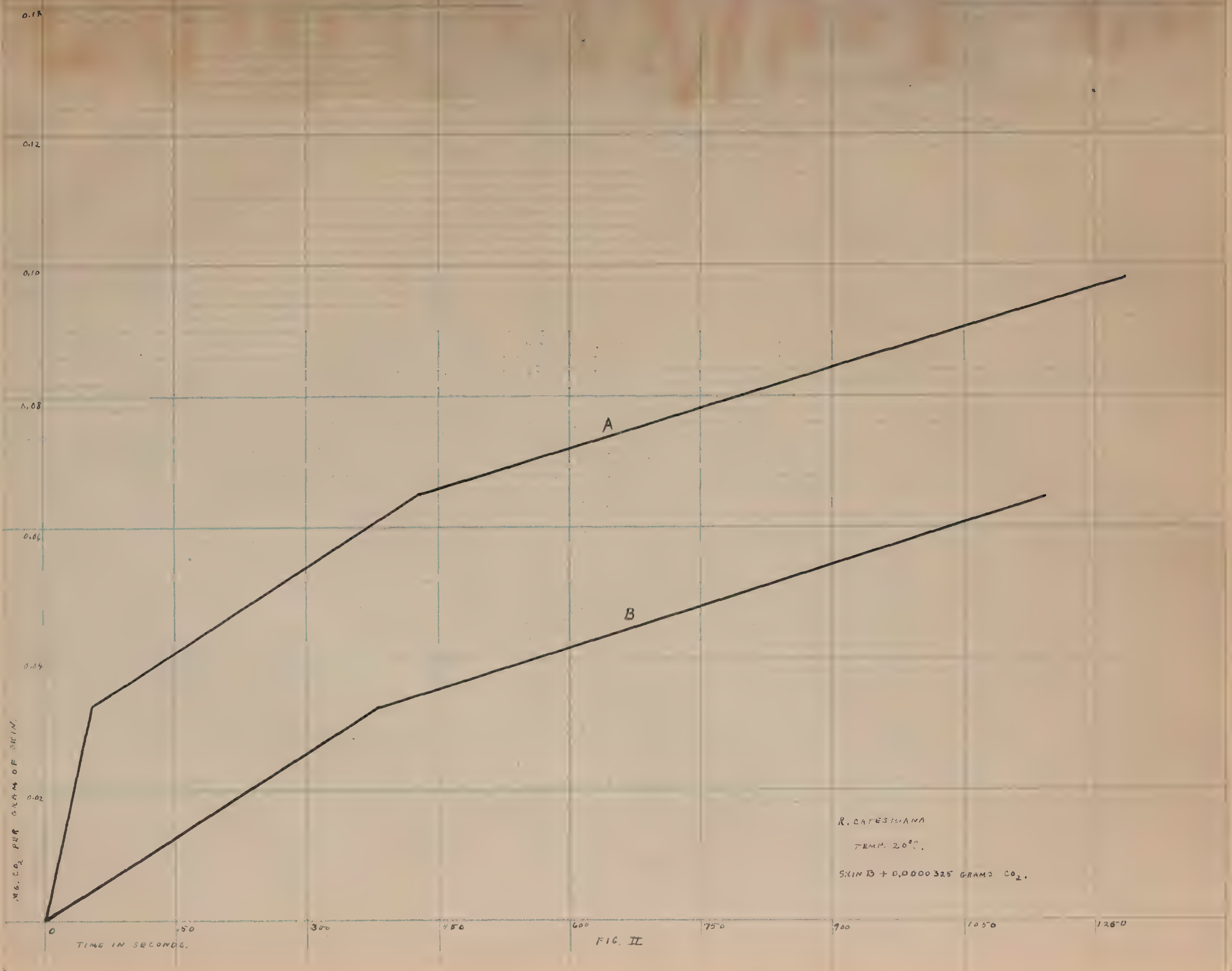

0.14
0.12
0.10
0.08
0.06
0.04
0.02
MG. CO$_2$ PER GRAM OF SKIN
0
150
300
450
600
750
900
1050
1200
TIME IN SECONDS
R. CATESBIANA
TEMP. 22°C.

FIG. I

long as did the experiment. This peculiarity is well shown in Fig. I. It seemed possible that the accumulation of the acid within the closed vial might be the cause of the retardation of further carbon dioxide production by the skin and a series of experiments was attempted in order to test the value of this suggestion.

Two pieces of skin were selected from the same animal. One piece was put in Ringer's solution as usual; the other was placed in a vial also containing Ringer's solution but to which a drop of carbon dioxide saturated water had been added, so that its color was exactly that of the first standard. The rates at which the two pieces of skin gave off carbon dioxide were then compared and it was found that the skin placed in the solution containing carbon dioxide produced this gas much more slowly than did the skin which was in ordinary Ringer's solution. Moreover, the rate of carbon dioxide production of the skin in the solution with a pH equal to that of the first standard was closely comparable to the rate at which the skin used as a control gave off this gas, after the pH of its solution, thru the accumulation of carbon dioxide produced by itself, had become the same as that of the first standard. The experiment was repeated with fourteen different frogs and the relation between the two rates was much the same in each case. The results of a typical experiment are shown in Fig. II.

It was calculated that the drop of carbon dioxide saturated water added to the Ringer's solution contained, according to the temperature, from 0.0000278 to 0.0000343 grams of carbon dioxide. The possibility that such a minute amount of a substance could produce such radical changes in the rate of respiratory metabolism appeared, at first that, highly improbable. But a more thoro consideration of the matter helped to eliminate this objection. The


0.14
0.12
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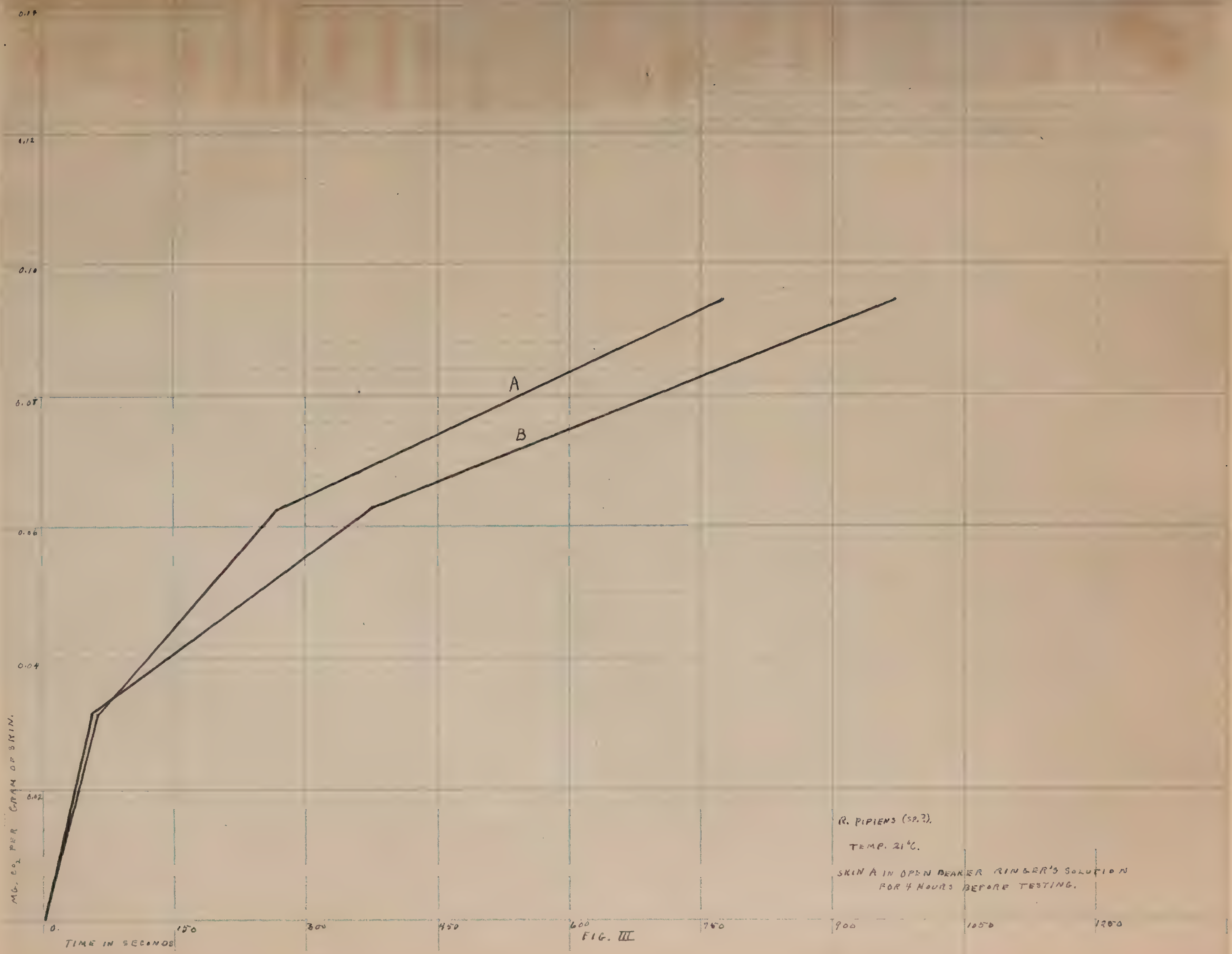
0.08
0.06
0.04
0.02
MG. CO$_2$ PER GRAM OF SKIN.
0
150
300
450
600
750
900
1050
1200
TIME IN SECONDS.
A
B
R. CATESBIANA
TEMP. 20°C.
SKIN B + 0.0000325 GRAMS CO$_2$.

FIG. II

amount of carbon dioxide added, even tho so small in itself, caused a considerable change in the pH of the solution. Physiological oxidations are supposed to be dependent on enzymes and enzymes are profoundly affected by slight changes in pH. Hence it is not at all unlikely that even small amounts of carbon dioxide, when not allowed to escape from the solution bathing the tissue, have a very significant effect on its rate of carbon dioxide production. The unusual physiological action of carbon dioxide itself must not be forgotten. According to Jacobs (2) its toxic effect is decidedly higher than that of other acid solutions of the same pH. This he explains by the fact that carbon dioxide in its gaseous state passes thru cell walls much more readily than substances of a more solid nature. Once inside the cell it reacts with the water there to form carbonic acid which, on dissociation, has a harmful effect on the protoplasm.

Might it not be that the decreasing rate of carbon dioxide production was due to the gradual death of the skin? In order to test this possibility a number of experiments were performed. A frog was pithed and skinned in the usual manner. The desired portion of skin was cut in half and one piece was placed in a vial and tested immediately; the other was allowed to remain in an open beaker of Ringer's solution for a considerable time - five hours in one case - and then tested in order to determine its rate of respiratory exchanges. When the results of the two procedures were compared it was found that there was almost no difference between them. Carbon dioxide was given off by the skin which had undergone the prolonged treatment with Ringer's solution at a rate which was remarkably close to that at which the skin newly removed from the animal's body had produced it. Such a condition is represented in Fig. III. The results of a number of experiments of this nature agreed so closely that little doubt remained as to the ability of the isolated skin,


0.14
0.12
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0.08
0.06
0.04
0.02
MG. CO$_2$ PER GRAM OF SKIN.
0.
150
300
450
600
750
900
1050
1200
TIME IN SECONDS
A
B
R. PIPIENS (SP.?).
TEMP. 21°C.
SKIN A IN OPEN BEAKER RINGER'S SOLUTION
FOR 4 HOURS BEFORE TESTING.

FIG. III

when properly treated, to maintain a nearly normal rate of carbon dioxide production over many hours. The death of the skin was thus eliminated as a possible explanation for the typical curves obtained by the use of this method.

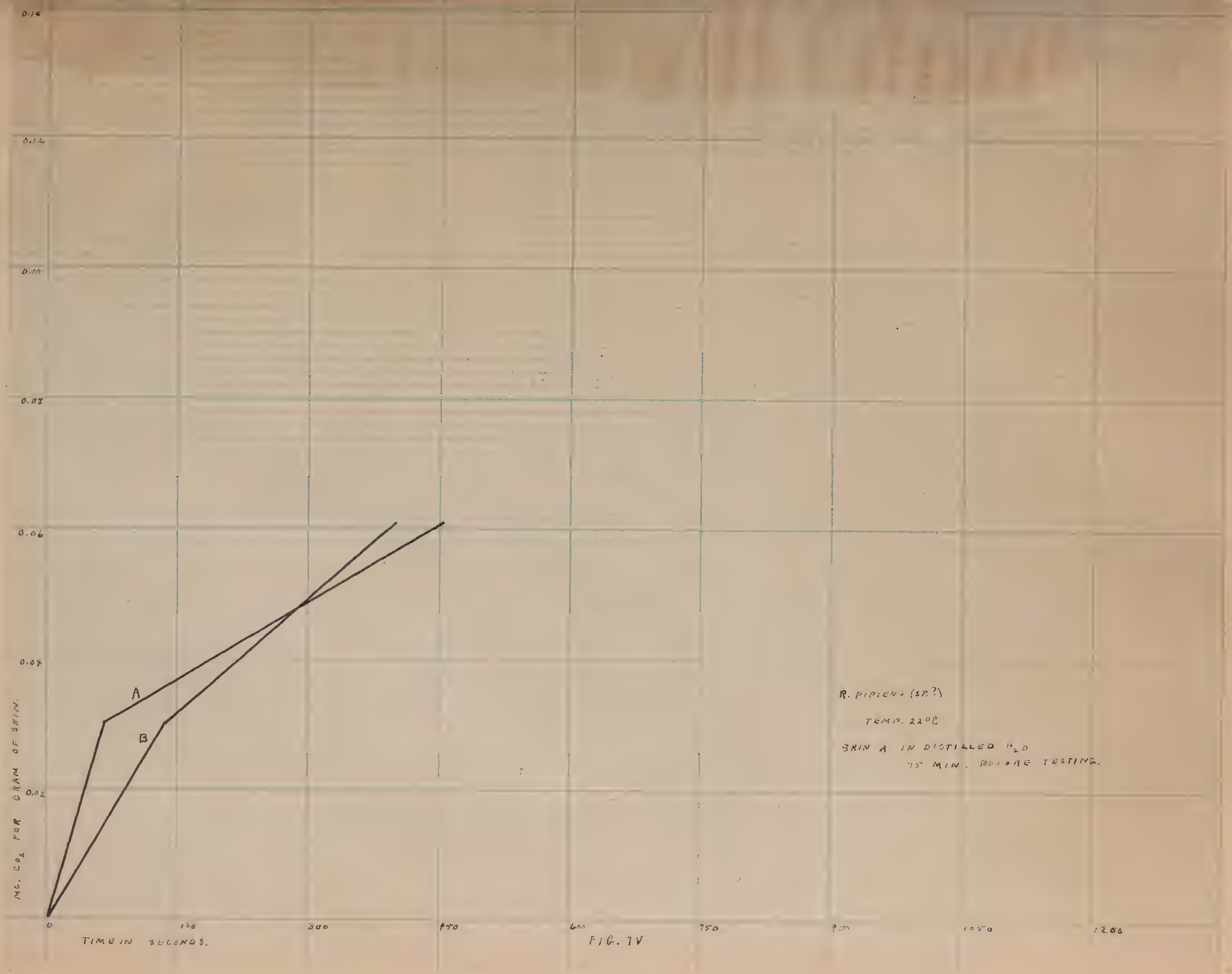
What would happen if the carbon dioxide, after causing this depression in the rate of respiratory metabolism, were removed? In order to answer this question pieces of skin which had been tested in closed vials and kept there until their rates of carbon dioxide production had fallen quite low, were placed in an open beaker of Ringer's solution (which was shaken occasionally so as to hasten the escape of the excess carbon dioxide) and allowed to remain there for ten minutes or more. After the skins had been thoroly washed in this way they were placed back in the vials (which had meanwhile been boiled out with distilled water) and retested. The resulting rates agreed so closely with the original rates that the ability of the skin to return to its normal condition, after the inhibiting factor had been removed, was quite apparent. In other words, the action of small amounts of carbon dioxide is a reversible one. This agrees well with the theory that oxidations, in living tissues, are enzymic in nature. Each enzyme, it is believed, works best at a definite and characteristic pH value. Slight variations above or below this value decrease its effectiveness, but unless the change is an extreme one the enzyme is capable of resuming its maximum ~~shiki~~ activity when the proper pH is restored. It seems likely that this explanation fits the case under discussion.

It is of interest to note at this point that G. H. Parker (3) has investigated the carbon dioxide output of dog-fish nerve with apparatus very similar to that used in the present series of studies. When his results, expressed in the form of graphs, are

examined it is fould that there is a remarkable resemblance between the curves produced in his experiments and those in the present ones. The initial "gush" of carbon dioxide, as he calls it, is soon followed by a much lower rate of production. Parker proposes several explanations for this but does not consider the possible effects of carbon dioxide accumulation. The suggestions offered in this paper might possibly account for such results.

The effects of distilled water on frog-skin were also investigated. Carbon dioxide production dropped to almost nothing when the skin was immersed in this liquid. In one experiment the standards were made up with, and the skins were tested in vials containing, besides the indicator, nothing but distilled water (which had been boiled in order to drive off any carbon dioxide dissolved in it, and then cooled). During the first five minutes the solution became slightly more acid but after that no perceptible change in color occurred, altho the experiment extended over many hours.

Can frog-skin recover from the effects of exposure to distilled water? The white skin from a frog was cut into two pieces; one was placed in an open beaker of Ringer's solution and the other in a similar vessel of distilled water. Here they remained for seventy-five minutes. At the end of this time the sink which had been in the distilled water was found to be unnaturally rigid and quite unlike the other skin in appearance. Each was removed, placed in a vial containing Ringer' solution and tested in the usual way. The skin which had been treated with distilled water gave off carbon dioxide at a *higher* rate than the control skin. After a short time the rate approached that of the normal tissue and then fell below it. The results are compared in Fig. IV. Only four experiments of this kind were performed, the results, however, being similar in



R. PIPIENS (SP.?)

TEMP. 22°C

SKIN A IN DISTILLED H_2O
75 MIN. BEFORE TESTING.

FIG. IV

each case. As yet no explanation for this phenomenon has been formulated.

The object of these experiments, at the outset, was to determine the rate at which frog-skin produces carbon dioxide. But if the conclusions drawn during the preceding discussion are correct - as at present they appear to be - it is safe to say that the actual rate of carbon dioxide production by such a tissue as frog-skin cannot be determined with the method here used. Apparatus is needed which will remove the carbon dioxide as fast as it is formed. It is probable that the normal rate of carbon dioxide production by frog-skin is most nearly approximated by figures which would be a little above the rate at which carbon dioxide is given off during the first few moments of the experiments just described.

(Following the summary a graphic representation of each experiment performed will be found.)

Summary.

1. Carbon dioxide, and no other acid, is given off, in amounts directly proportional to its weight, by frog-skin immersed in Ringer's solution.

2. The carbon dioxide produced by the skin, when not allowed to escape, retards the further production of carbon dioxide.

3. Frog-skin, in Ringer's solution, when the carbon dioxide given off by it is allowed to escape, maintains a nearly normal rate of carbon dioxide production over many hours.

4. Skin, which is producing carbon dioxide at a decreased rate because of the presence of small quantities of carbon dioxide can be restored to its normal condition by the removal of the carbon dioxide.

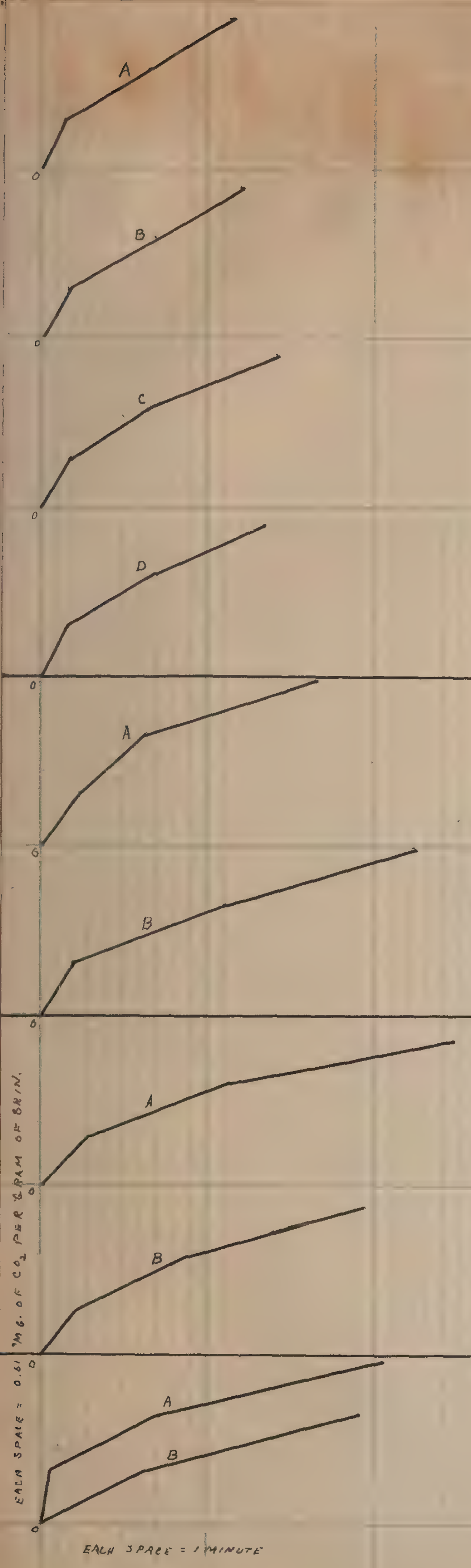
5. Distilled water causes an almost complete cessation of carbon dioxide production by frog-skin. This effect, on the removal of the inhibiting factor, also appears to be reversible.

References.

1. Osterhout, W. J. V. (1918). A Method of Studying Respiration. Jour. of Gen. Physiol., 1, 17.

2. Jacobs, M. H. (1920). To What Extent are the Physiological Effects of Carbon Dioxide Due to the Hydrogen Ion? Am. Jour. Physiol., 51, 321.

3. Parker, G. H. (1925). The Production of Carbon Dioxide by Nerve. Jour. of Gen. Physiol., 7, 641.



EXP. I

R. CATESBIANA

TEMP. 22°C.

SKIN C SAME SKIN AS A RETESTED $3\frac{1}{2}$ HOURS LATER.
" D " " " B " " " "

EXP. II

R. CATESBIANA

TEMP. 19°

EXP. III

R. CATESBIANA

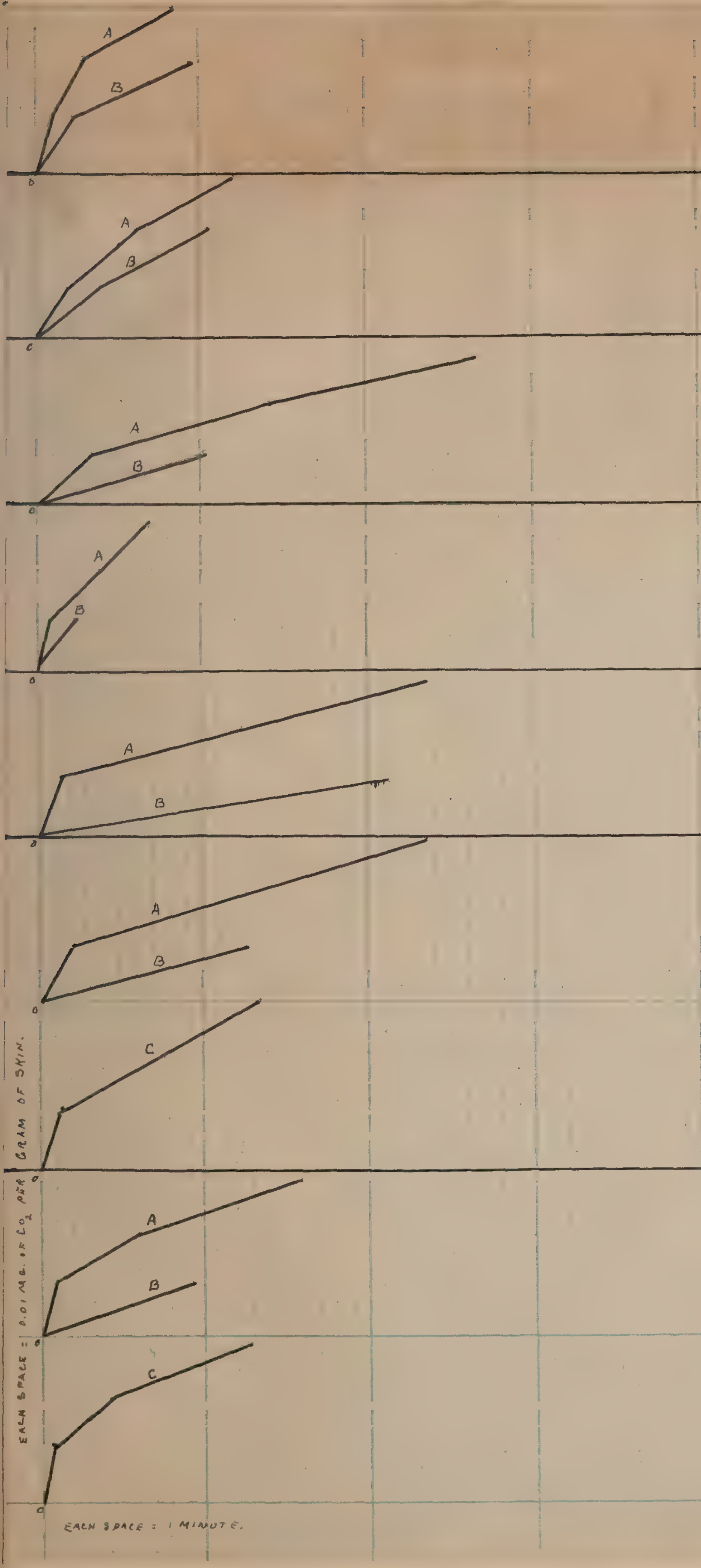
TEMP. 23°C.

EXP. IV

R. CATESBIANA

TEMP. 26°C

SKIN B IN SOLUTION WITH 0.0000325 G.M. CO_2.



EXP. V

R. PIPIENS (SP.?)

TEMP. 19°C

SKIN B WITH 0.0000336 GM. CO_2 IN SOLUTION.

EXP. VI

R. CATESBIANA

TEMP. 21°C.

SKIN B WITH 0.0000325 GM. CO_2 IN SOLUTION.

EXP. VII

R. CATESBIANA

TEMP. 22°C.

SKIN B WITH 0.0000606 GM. CO_2 IN SOLUTION.

EXP. VIII

R. CATESBIANA

TEMP. 23°C.

SKIN B WITH 0.0000.592 GM CO_2 IN SOLUTION.

EXP. IX

R. PIPIENS (SP.?)

TEMP. 20°C.

SKIN B WITH 0.000065 GM. CO_2, IN SOLUTION.

EXP X

R. PIPIENS (SP.?)

TEMP. 19°C.

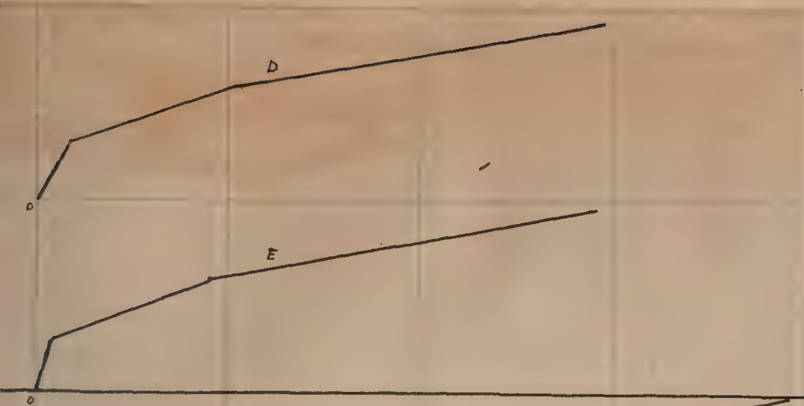
SKIN B WITH 0.0000672 GM. CO_2 IN SOLUTION.
SKIN C KEPT IN OPEN BEAKER RINGER'S SOLUTION
FOR 3½ HOURS.

EXP. XI

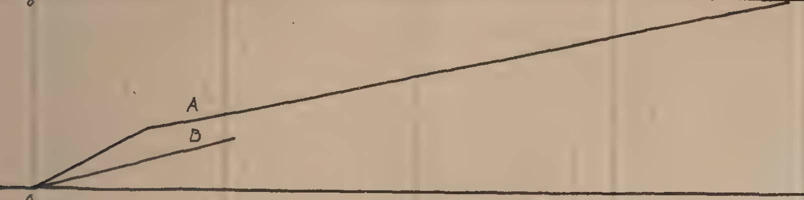
R. PIPIENS (SP.?)

TEMP. 21°C

SKIN B IN SOLUTION WITH 0.000063 GM CO_2.
SKIN C KEPT IN OPEN BEAKER OF RINGER'S
SOLUTION 4 HOURS BEFORE TESTING.



SKIN D SAME SKIN AS A RETESTED.
" E " " " B " (WITHOUT EXTRA CO_2).



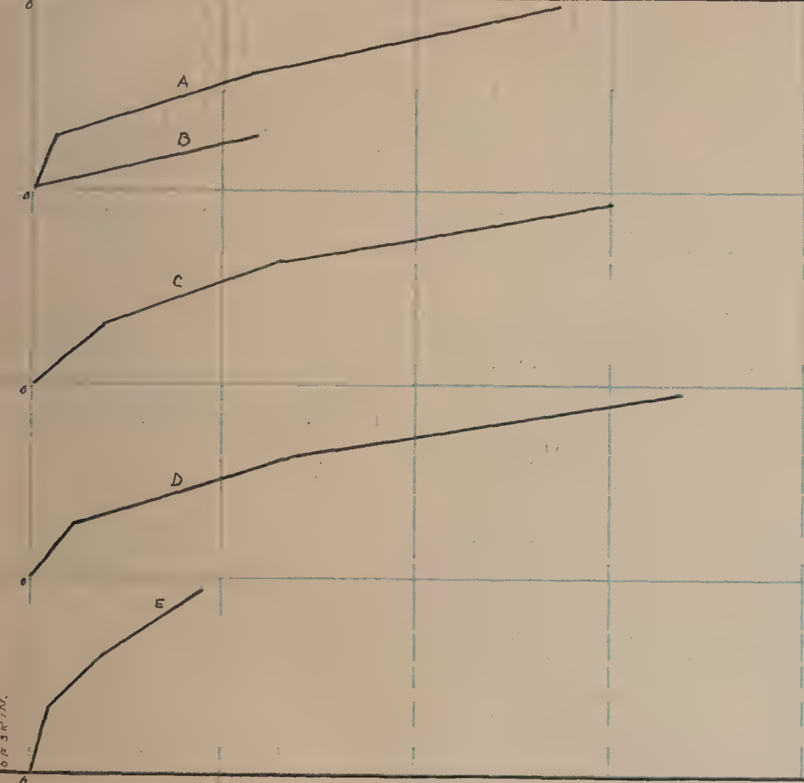
EXP. XII

R. PIPIENS (SP.?)

TEMP. 19°C.

SKIN SUSPENDED ABOVE LIQUID, STUCK TOGETHER IN SMALL MASS.

SKIN B TESTED IN VIAL WITH 0.0000672 GM. CO_2



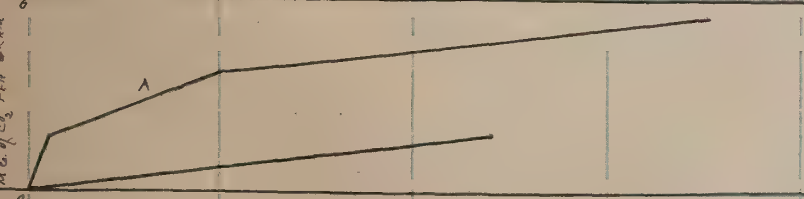
EXP. XIII

R. PIPIENS (SP.?)

TEMP. 21°C.

SKIN B IN SOLUTION WITH 0.000063 GM. CO_2.
SKIN C SAME SKIN AS A RETESTED.
" D " " " B " (WITHOUT EXTRA CO_2)

SKIN E KEPT IN OPEN BEAKER RINGER'S SOLUTION 3 HOURS BEFORE TESTING.

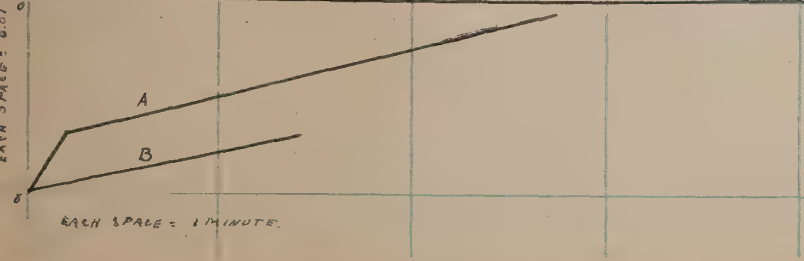


EXP. XIV

R. PIPIENS (SP.?)

TEMP. 22°C.

SKIN B WITH 0.0000606 GM. CO_2 IN SOLUTION

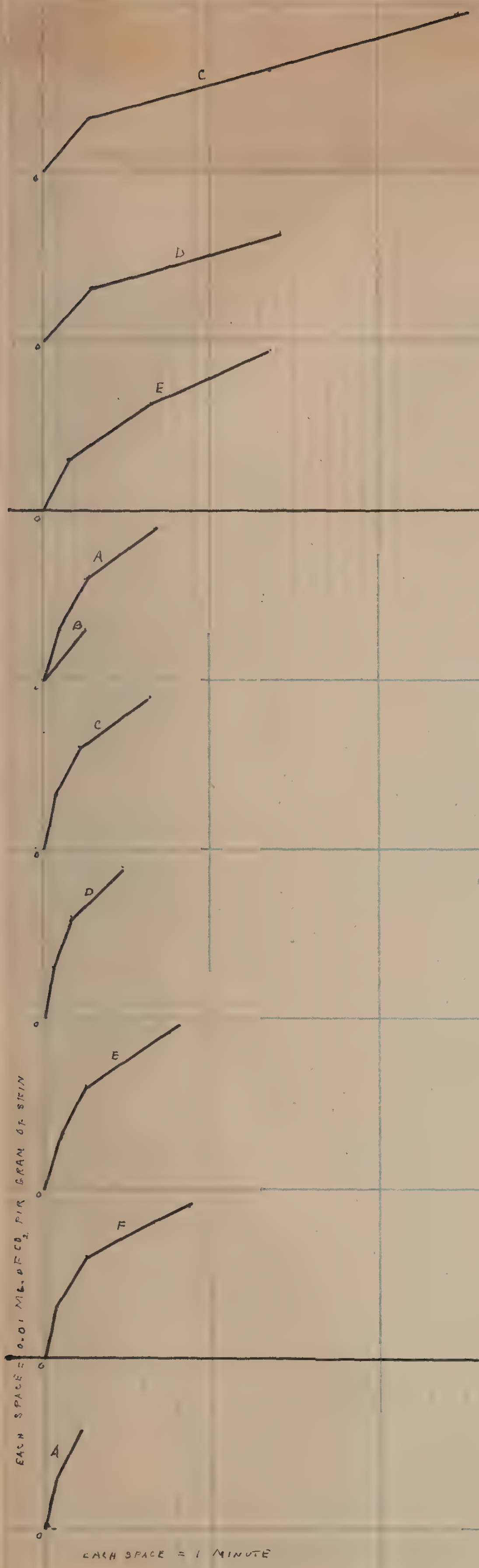


EXP. XV

R. PIPIENS (SP.?)

TEMP. 21°C.

SKIN B IN SOLUTION WITH 0.000063 GM. CO_2



SKIN C SAME SKIN AS A RETESTED.
" D " " " B " (WITHOUT EXTRA CO_2)
SKIN E KEPT IN OPEN BEAKER OF RINGER'S SOLUTION
FOR 3½ HOURS BEFORE TESTING.

EXP. XVI

R. PIPIENS (SP?)

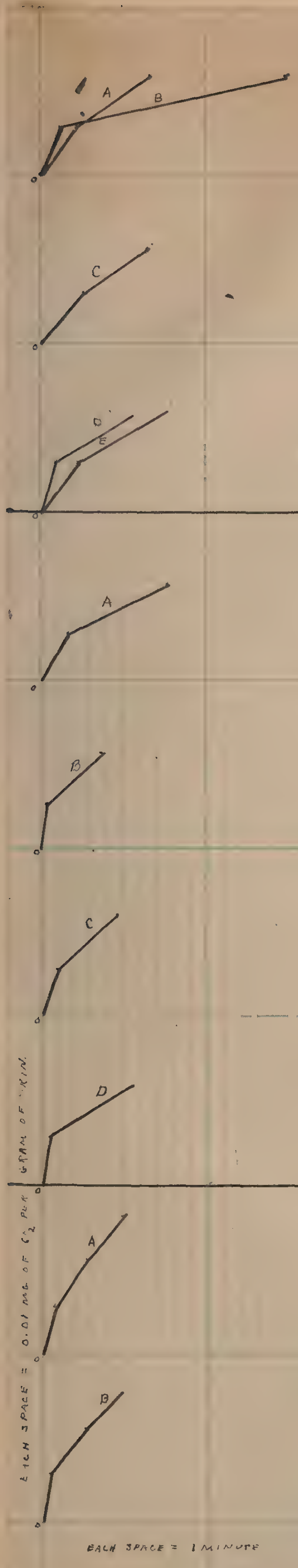
TEMP. 28°C

SKIN B WITH 0.0000592 GM. CO_2 IN SOLUTION.
SKIN C SAME SKIN AS A RETESTED.
" D " " " B " (WITHOUT EXTRA CO_2)
" E " " " C "
" F " " " D " (WITHOUT EXTRA CO_2)

EXP. XVII

R. PIPIENS (SP?)

TEMP. 25°C.



EXP. XVIII

R. PIPIENS (SP?)

TEMP. 22°C

SKIN A IN RINGER'S SOLUTION 75 MIN, BEFORE TESTING
" B " DISTILLED H_2O " " " "
SKIN C . 0.65% NACL SOLUTION " " " "
" E " RINGER'S SOLUTION 5 HOURS, " "
" D " DISTILLED H_2O " " " "

EXP. XIX

R. PIPIENS (SP.?)

TEMP. 23°C.

SKIN A IN RINGER'S SOLUTION FOR 25 MIN. BEFORE TESTING
" B " DISTILLED H_2O " " " " "
" C " RINGER'S SOLUTION " 3 HOURS " "
" D " DISTILLED H_2O " " " " "

EXP. XX

R. PALUSTRIS

TEMP. 25°C

SKIN A IN RINGER'S SOLUTION FOR 50 MIN. BEFORE TESTING
" B " DISTILLED H_2O " " " " "
THEN IN RINGER'S FOR 10 MIN.

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ANNEX

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